Pharmacokinetics of dalbavancin in plasma and skin blister fluid

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Objectives: Dalbavancin is a novel lipoglycopeptide antibiotic in development for the treatment of complicated skin and skin structure infections (cSSSIs) caused by Gram-positive bacteria. The aim of the present study was to assess the penetration of dalbavancin into skin blister fluid.

Methods: Nine healthy subjects (five males; ranging in age from 26 to 57 years) were administered a single 30 min intravenous infusion of dalbavancin at a dose of 1000 mg. Skin blisters were induced by application of cantharidin ointment. Plasma and blister fluid samples were collected over 7 days post-dose, and concentrations of dalbavancin were assessed by a validated LC/MS/MS assay. Pharmacokinetics were determined by non-compartmental methods, and drug penetration was assessed based on the ratio of area under the curve (AUC) in the blister fluid versus plasma for each subject.

Results: The mean (SD) peak concentration of dalbavancin in plasma and blister fluid was 285 (31.1) and 67.3 (18.2) mg/L, respectively; the corresponding AUCDay 7 values were 10 806 (1926) and 6438 (1238) mg.h/L, respectively. The mean (SD) penetration of dalbavancin into blister fluid was 59.6% (6.3%). By Day 7, the mean concentration of dalbavancin in plasma and blister fluid was 46.5 and 30.3 mg/L, respectively.

Conclusions: Dalbavancin concentrations in blister fluid remained well above the MIC90 values for pathogens commonly implicated in cSSSIs such as Staphylococcus aureus, including methicillin-resistant S. aureus (MIC90 = 0.06 mg/L) and β-haemolytic streptococci (MIC90 = 0.03 mg/L) through Day 7. These pharmacokinetic data support the use of dalbavancin in the treatment of cSSSIs caused by susceptible Gram-positive pathogens.

Keywords: lipoglycopeptides, infections, cSSSIs

Introduction

Dalbavancin is a novel lipoglycopeptide antibiotic in late stage clinical development for the treatment of serious infections, including skin and skin structure infections. Dalbavancin, like other glycopeptides, inhibits cell-wall peptidoglycan cross-linking by binding to the terminal of the D-alanyl-D-alanine pentapeptide chain in nascent peptidoglycan.1 It has excellent in vitro activity against a broad range of Gram-positive bacteria, including methicillin-resistant Staphylococcus aureus (MRSA), and is generally more active in vitro than vancomycin.2 Dalbavancin’s MIC values for staphylococci, including S. aureus and coagulase-negative staphylococci of various species, range from ≤0.015 to 0.5 mg/L (MIC90 = 0.06 mg/L).

Dalbavancin MIC values for streptococci range from ≤0.015 to 0.25 mg/L (MIC90 = 0.03 mg/L).3–5

A previous study conducted in healthy volunteers demonstrated that the maximum concentrations of dalbavancin (Cmax) are achieved immediately following the end of an intravenous (iv) infusion. The volume of distribution at steady-state is ~8–12 L.6 The eventual distribution of the drug to the tissues, however, appears to be more extensive. A quantitative distribution study in rats showed that concentrations of drug-derived radioactivity in tissues, including skin, were comparable to those observed in plasma.7

In healthy volunteer studies, total clearance of dalbavancin was estimated to be 0.04 L/h.8 The drug is eliminated in both urine and faeces, with the majority eliminated as intact drug and...
a small fraction of the dose eliminated as OH-dalbavancin. An elimination half-life of ~1 week characterizes the majority of drug distribution. Because of its pharmacokinetic profile, a single 1000 mg dose on Day 1 followed by 500 mg on Day 8 provides plasma concentrations that greatly exceed MICs for target organisms during the dosing interval of 1 week, supporting a once weekly administration.

Because skin infections occur in the extravascular space, antibiotic pharmacokinetics are increasingly being studied in blister fluid as a surrogate for the disposition of the drug into this infection site. Antimicrobial distribution studies such as blister fluid studies, in combination with pivotal trial data, have been suggested to support new drug applications for skin and skin structure infections. In an attempt to gather these data, a preliminary assessment of dalbavancin’s penetration into blister fluid was undertaken in two healthy subjects participating in a dose escalation study using a limited blister fluid sampling scheme. As a result of this methodology, penetration was calculated based on the ratio of the drug concentration in blister fluid to plasma at specific sampling times as opposed to the conventional method of comparing the ratio of area under the concentration–time curve for the two matrices. Data derived from these two subjects revealed a penetration ratio of 0.83–1.11 for dalbavancin.

As a result of both the limited number of subjects and sample collection scheme utilized in the above noted study, the current trial was conducted in healthy subjects to fully assess the penetration of dalbavancin into cantharidin-induced skin blister fluid.

Methods

A single-dose, open-label, single-centre study was performed in healthy volunteers, which evaluated the pharmacokinetic profile and skin penetration of dalbavancin. The study enrolled healthy subjects between 19 and 65 years of age and were within −10% to +50% of their ideal body weight for their sex, height and body frame. Female subjects were to be surgically sterile, at least 2 years post-menopausal, or, if sexually active, were to have agreed to utilize an approved form of contraception. Exclusion criteria included any evidence or history of clinically significant abnormalities, a positive test for human immunodeficiency virus (HIV) antibody, a positive test for hepatitis B or C virus, a positive test for drugs of abuse, history of drug or alcohol abuse, or the use of any drug that might affect hepatic microsomal enzyme activity.

All subjects provided written informed consent. The study was approved by the Institutional Review Board (MDS Pharma Services) and was conducted in accordance with the Declaration of Helsinki, The International Conference on Harmonization, Good Clinical Practices, and the Food and Drug Administration regulations (21 CFR parts 50 and 56) for the protection of the rights and welfare of human subjects participating in biomedical research.

Subjects received a single, 30 min iv infusion of 1000 mg of dalbavancin on study Day 1. Blistered skin infections were induced by applying 0.2 mL of an ointment containing 0.25% cantharidin powder (Sigma Laboratories, St Louis, MO, USA) and standard ointment base to the skin of the anterior forearm. A unique blister was formed for each sample time. To protect the integrity of the blister, a plastic cup was placed around each ointment application and the forearm was wrapped in a protective bandage. The cantharidin ointment remained in contact with the skin for ~12–14 h. Blister fluid from each subject was sampled just prior to drug infusion, 12 h following the start of infusion, and on study Days 3, 5 and 7 approximately ±30 min relative to time of initiation of drug infusion on Day 1.

Blood samples (10 mL each) were drawn from an indwelling catheter into heparinized tubes. Blood samples occurring during the blister fluid collection period were obtained prior to dose, at the end of infusion (+15 min), and 12 h post-start of infusion and on study Days 2, 3 and 7. Plasma was separated and transferred into labelled polypropylene screw cap transfer tubes and stored at −20°C until assayed.

A validated liquid chromatography coupled to tandem-mass spectrometry (LC/MS/MS) method was used for the determination of dalbavancin and its hydroxyl-metabolite (OH-dalbavancin) in human plasma at Prevalere Life Sciences, Inc., Whitesboro, NY, USA. The LC/MS/MS method for dalbavancin in plasma was validated in the linear concentration range of 1.0–128 μg/mL for dalbavancin and 0.4–12.8 μg/mL for OH-dalbavancin. The range was further extended by dilution. Assay validations included long-term and freeze/thaw stability that were adequate to meet the needs of this study. Overall precision for the quality control samples in plasma, as measured by %RSD (where RSD stands for relative standard deviation), was <14.4% for dalbavancin and <17.9% for OH-dalbavancin. Overall accuracy, as measured by %RE (where RE stands for relative error), for these quality control samples ranged from 2.7% to 2% for dalbavancin and from 12.0% to 19.0% for OH-dalbavancin. Blister fluid samples were analysed for dalbavancin and OH-dalbavancin using the validated human plasma LC/MS/MS method. A cross-validation using standards and quality controls prepared in blister fluid was successfully performed prior to sample analysis.

Safety and tolerability were monitored throughout the study with laboratory tests, physical examinations that included vital signs, electrocardiograms and adverse event (AE) monitoring. During the blister fluid collection interval, safety evaluations were performed on Days 1 and 7. Subjects were queried about possible AEs and symptoms daily, and any reported AE was assessed by severity and relationship to study drug.

The pharmacokinetic parameters for dalbavancin in plasma and blister fluid were estimated by non-compartmental methods using WinNonlin (Version 4.0, Pharsight Corporation, Mountain View, CA, USA). The pharmacokinetic parameters included the maximum observed plasma concentration (Cmax) and area under the concentration versus time curve through Day 7 (AUCDay 7). Cmax was obtained directly from the data, and AUC was calculated using the linear-trapezoidal rule. The degree of penetration of dalbavancin into the skin was determined by the ratio of the AUC of skin blister fluid through Day 7 to that of the plasma through Day 7.

Results

Nine healthy subjects were enrolled and completed all study assessments. There were five male subjects and four female subjects. Subjects ranged in age from 26 to 57 years, with a median age of 37. Eight (88.9%) subjects were Caucasian and one (11.1%) subject was American Indian. All subjects were within −10% to +50% of their ideal deal body weight for their sex, height and body frame. Data for all nine subjects were included in the analysis. Mean concentrations of dalbavancin in plasma and blister fluid following administration of a single 1000 mg dose of dalbavancin are shown in Figure 1. In both plasma and blister fluid, concentrations of OH-dalbavancin were at or below the levels of assay detection. Individual subject dalbavancin
pharmacokinetic parameters and summary statistics are given in Table 1. Plasma $C_{\text{max}}$ was reached shortly after the end of infusion and was $\sim 285$ mg/L. The mean plasma $\text{AUC}_{\text{Day 7}}$ was $10,806 (\pm 1,926)$ mg h/L and had a coefficient of variation (CV) of $20\%$.

Dalbavancin concentrations in blister fluid for all subjects remained well above the MIC$_{90}$ of $S.\ aureus$ and streptococci through Day 7. The mean blister fluid $\text{AUC}_{\text{Day 7}}$ was 6438 (±1238) mg h/L. Mean penetration of dalbavancin into blister fluid was $\sim 60\%$. Similar to the pharmacokinetic parameters in plasma, low intersubject variability was observed across the blister fluid parameters.

Dalbavancin was well tolerated among all subjects and did not result in any premature discontinuations from the study. There were no deaths or serious AEs in the study. All nine subjects reported at least one treatment-emergent AE, the majority of which (20/23, 87%) were mild in intensity. The remaining three AEs (13%) were moderate in intensity, and there were no AEs of severe intensity during the study. The most frequently reported AE was infusion site pain (four subjects), followed by infusion site erythema, headache and sinus congestion (two subjects each). All other AEs (namely, back pain, constipation, cough, ear infection, eye pruritus, kerato conjunctivitis sicca, loose stools, nausea, sneezing and vomiting) were each reported by one subject. Among these AEs, infusion site pain, infusion site erythema, loose stools and nausea were considered possibly or probably related to study drug.

### Discussion

The degree of penetration of dalbavancin into skin blister fluid was re-assessed in the present study. Unlike the preliminary trial in two subjects, this study utilized a sufficient number of subjects, a dose that has been employed in clinical trials and a sampling scheme that allowed for an adequate characterization of penetration using the ratio of the AUC–time curve for the two matrices. As a result of these disparities in methodology, it is not surprising that an apparent difference in the penetration ratios has been observed between the datasets. Although the current study reports a penetration ratio of 60% versus the 83% to 111% observed in the two previously studied healthy subjects, our achievable concentrations in blister fluid appear sufficient relative to the MICs for Gram-positive pathogens, such as $S.\ aureus$ and streptococci.

The assay in this study measured total dalbavancin, and the data presented are not corrected for protein binding. The binding of dalbavancin to plasma proteins, preferential to albumin, is reversible and $\sim 93\%$. As such, it is not well understood as to whether free or total drug concentrations are the best measure for the prediction outcomes with dalbavancin, and only limited data are available on whether concentration- or time-dependent pharmacodynamic parameters will best forecast efficacy.

Correcting for a 7% free fraction of dalbavancin, the ratio of the total (bound plus unbound) AUC in blister fluid versus the free (unbound) drug AUC in plasma is $\sim 8.5$. Similar evaluations have been conducted for telavancin (following a 7.5 mg/kg once daily regimen for three doses) and oritavancin (following a single 800 mg iv dose), and have shown drug penetration in the

### Table 1. Pharmacokinetic parameters of dalbavancin in plasma and blister fluid

<table>
<thead>
<tr>
<th>Subject</th>
<th>$C_{\text{max}}$ (mg/L)</th>
<th>$\text{AUC}_{\text{Day 7}}$ (mg h/L)</th>
<th>Day 7 concentration (mg/L)</th>
<th>$C_{\text{max}}$ (mg/L)</th>
<th>$\text{AUC}_{\text{Day 7}}$ (mg h/L)</th>
<th>Day 7 concentration (mg/L)</th>
<th>degree of penetration (%)</th>
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blister fluid of ~40% and 19%, respectively. In these studies, drug protein binding in blister fluid was not determined but was assumed to be similar to that in plasma. The plasma protein binding of telavancin and oritavancin is ~95% and 87%, respectively. We used the mean AUC values reported for telavancin and oritavancin (800 mg dose), and corrected them for the free drug fraction (5% and 13%, respectively). Based on these calculations, the ratio of the total (bound plus unbound) AUC in blister fluid versus the free (unbound) drug AUC in plasma is ~8.4 and 1.4 for telavancin and oritavancin, respectively. This ratio is largely dependent on the correction used for protein binding. In this regard, dalbavancin appears to be similar to telavancin, largely due to similar protein binding. The estimated free fraction for oritavancin (13%) is much higher than that for dalbavancin (7%) and oritavancin (5%), respectively, which presumably explains the difference in this ratio. Finally, it should be noted that based on total drug AUC in both plasma and blister fluid, the penetration into blister fluid is higher for dalbavancin (60%) compared with telavancin (40%) and oritavancin (19%). Regardless, however, this study demonstrates the ability of dalbavancin to penetrate into the skin at concentrations, free or total, well above the MICs for clinically relevant Gram-positive pathogens through the 1 week post-dose period.

Results from Phase II and Phase III studies show dalbavancin to be a useful alternative in the treatment of skin and soft tissue infections caused by susceptible Gram-positive pathogens and the data from the current study support these observations.

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Transparency declarations

D. P. N. and H. K. S. have ‘none to declare’. E. S., M. B. and J. A. D. work for the sponsor that supported the study.

References